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Conformational States of *N*-Acylalanine Dithio Esters: Correlation of Resonance Raman Spectra with Structures†

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ABSTRACT: The conformational states of *N*-acylalanine dithio esters, involving rotational isomers about the RC(=O)NH—CH(CH_3) and NHCH(CH_3)—C(=S) bonds, are defined and compared to those of *N*-acylglycine dithio esters. The structure of *N*-(*p*-nitrobenzoyl)-DL-alanine ethyl dithio ester has been determined by X-ray crystallographic analysis; it is a B-type conformer with the amide N atom cis to the thiol sulfur. Raman and resonance Raman (RR) measurements on this compound and for the B conformers of solid *N*-benzoyl-DL-alanine ethyl dithio ester and *N*-(β-phenylpropionyl)-DL-alanine ethyl dithio ester and its NHCH(CD_3)C(=S) and NHCH(CH_3)^{13}C(=S) analogues are used to set up a library of RR data for alanine-based dithio esters in a B-conformer state. (Methyloxycarbonyl)-L-phenylalanyl-L-alanine ethyl dithio ester crystallizes in an A-like conformational state wherein the alanine N atom is nearly cis to the thiono S atom (C=S) [Varughese, K. I., Angus, R. H., Carey, P. R., Lee, H., & Storer, A. C. (1986) *Can. J. Chem. 64*, 1668-1673]. RR data for this solid material in its isotopically unsubstituted and CH(CD_3)C(=S) and CH(CH_3)^{13}C(=S) forms provide information on the RR signatures of alanine dithio esters in A-like conformations. RR spectra are compared for the solid compounds, for *N*-(*p*-nitrobenzoyl)-DL-alanine, *N*-(β-phenylpropionyl)-DL-alanine, and (methyloxycarbonyl)-L-phenylalanyl-DL-alanine ethyl dithio esters, and for several ^{13}C=S- and CD_3-substituted analogues in CCl_4 or aqueous solutions. The RR data demonstrate that the alanine-based dithio esters take up A, B, and C_5 conformations in solution. The RR spectra of these conformers are clearly distinguishable from those for the same conformers of *N*-acylglycine dithio esters. However, the crystallographic and spectroscopic results show that the conformational properties of *N*-acylglycine and *N*-acylalanine dithio esters are very similar.

Resonance Raman (RR) data for *N*-acylglycine dithioacyl papains have provided detailed information on the conformation of the covalently bound substrates (Carey & Storer, 1984, 1985). Spectral interpretation for these enzyme–substrate intermediates has been greatly aided by the study of model compounds in the form of *N*-acylglycine ethyl dithio esters. Many of the model compounds could be crystallized as single crystals suitable for X-ray crystallographic analysis, and since the crystals could also be examined by Raman and RR spectroscopy, it was possible to construct a library of precise structure–spectra correlations for use in interpreting the dithioacyl papain RR spectra.

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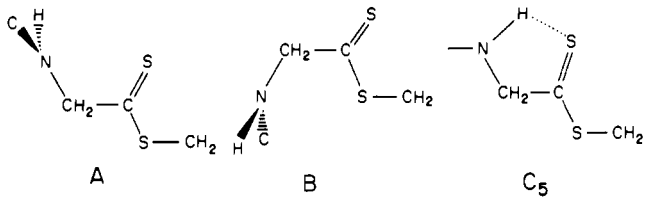

FIGURE 1: Conformers A, B, and C_5.

For every *N*-acylglycine dithio ester characterized, the conformation was found to be s-cis about the C(=S)—SC single bond. However, rotational isomers were found involving the C(=O)NH—CH_2C(=S) and NHCH_2—CS single bonds. These torsional angles are designated ϕ' and ψ', respectively, by analogy to the Ramachandran angles. In one conformational category, named conformer B, ϕ' is in the −70 to −100° region and ψ' is in the +10 to −25° range. In a second class, named conformer A, ϕ' is near −80° but ψ' is close to 160°. As can be seen in Figure 1, conformer B involves close approach of the amide N and thiol S atoms, while in conformer A the N atom is in close contact with the thiono S atom. A third conformational state, designated C_5 and usually found in solution in non-hydrogen-bonding solvents, is similar to conformer A except that $\phi' \approx 180°$, so that an NH to thiono S H-bond is formed within a five-membered ring.

Since glycine is an atypical amino acid in that it has a relatively unhindered conformational space (Schulz & Schirmer, 1979), we have begun to extend our RR studies to substrates with a side chain on the α carbon. In this paper we discuss the conformational preferences of *N*-acylalanine ethyl dithio esters using both crystallographic and spectroscopic data and show that the conformations taken up by these model compounds are very similar to those for *N*-acylglycine ethyl dithio esters.

Experimental Procedures

Materials

The D and L mixtures of *p*-$NO_2C_6H_4$C(=O)NHCH(CH_3)C(=S)SC_2H_5, $C_6H_5CH_2CH_2$C(=O)NHCH(CH_3)C(=S)SC_2H_5, $C_6H_5CH_2CH_2$C(=O)NHCH(CH_3)^{13}C(=S)SC_2H_5, $C_6H_5CH_2CH_2$C(=O)NHCH(CD_3)C(=S)SC_2H_5, CH_3OC(=O)NHCH($CH_2C_6H_5$)C(=O)NHCH(CH_3)^{13}C(=S)SC_2H_5, and CH_3OC(=O)NHCH($CH_2C_6H_5$)C(=O)NHCH(CD_3)C(=S)SC_2H_5 were prepared from their respective nitriles according to procedures previously described (Carey et al., 1984a; Angus et al., 1985). The *p*-nitro and β-phenylpropionyl nitriles were synthesized by reacting their respective acid chlorides with α-aminopropionitrile produced in situ (Angus et al., 1985). The *N*-(methyloxycarbonyl)-phenylalanyl-DL-alanine nitrile was synthesized by using a mixed anhydride procedure with *N*-(methyloxycarbonyl)-phenylalanine (Carey et al., 1984a) and α-aminopropionitrile produced in situ (Varughese et al., 1986).

For the synthesis of the ^{13}C- and CD_3-substituted nitriles, sodium [^{13}C]cyanide (99 atom %) or acetaldehyde-d_3 (99 atom %) was used, respectively (Merck, Sharpe and Dohme, Canada) (Angus et al., 1985).

The purity of the dithio esters was checked by NMR and elemental analysis, where the results agreed, within acceptable limits (±0.02 times the calculated percentage), with the theoretical values.

Methods

Yellow crystals of *N*-(*p*-nitrobenzoyl)-DL-alanine ethyl dithio ester were obtained by diffusing hexane into a solution of the dithio ester in ether: $C_{12}H_{14}N_2O_3S_2$, space group *Pbca*, a = 9.781 (1) Å, b = 21.917 (1) Å, c = 13.823 (1) Å, fw 298.4, ρc = 1.338 g cm^{-3}, λ(Cu Kα) = 1.5418 Å, μ(Cu Kα) = 32.1 cm^{-1}, and z = 8.

Table I: Positional and Thermal Parameters of Non-Hydrogen Atoms[a]

	x	y	z	$B(E)$
S(1)	84420 (25)	4729 (6)	78953 (12)	10.7
S(2)	96015 (16)	8366 (6)	59938 (12)	8.1
O(1)	10563 (2)	−878 (1)	7199 (2)	5.5
O(2)	9488 (7)	−3119 (2)	10607 (4)	13.4
O(3)	8121 (4)	−2515 (2)	11301 (3)	10.1
N(1)	8300 (3)	−717 (1)	7112 (2)	4.4
N(2)	8810 (5)	−2664 (2)	10606 (3)	7.6
C(1)	8229 (11)	1649 (4)	8433 (11)	17.0
C(2)	9012 (15)	1261 (4)	8097 (9)	18.2
C(3)	8852 (4)	356 (2)	6703 (3)	5.7
C(4)	8441 (4)	−276 (2)	6339 (3)	5.2
C(5)	7124 (6)	−259 (3)	5758 (4)	8.7
C(6)	9400 (3)	−990 (1)	7485 (2)	4.3
C(7)	9164 (3)	−1436 (1)	8289 (2)	4.3
C(8)	8193 (4)	−1340 (2)	8997 (3)	5.1
C(9)	8080 (4)	−1735 (2)	9764 (3)	5.9
C(10)	8926 (4)	−2239 (2)	9793 (3)	5.6
C(11)	9863 (5)	−2354 (2)	9079 (4)	6.5
C(12)	10002 (4)	−1943 (2)	8345 (3)	6.0

[a] $B(E)$ is calculated as $(8\pi^2/3)(U_{11} + U_{22} + U_{33})$. The coordinates are multiplied by 10^5 for sulfur atoms and by 10^4 for the rest.

All the crystallographic measurements were carried out by using a crystal of dimensions 0.6 × 0.25 × 0.20 mm^3, with a CAD4 diffractometer and Ni-filtered Cu Kα radiation. The unit cell dimensions were determined by using 25 well-centered reflections in the 2θ range of 54°–80°. The intensity data were measured to a 2θ limit of 150° by scanning $\omega/2\theta$ $\Delta\omega$ = 0.90 + 0.14 tan θ (DEG), aperture 4.0 + 0.3 tan θ (mn). The intensities of the reflections were monitored by using three standard reflections, and there were no systematic changes in the intensities. An empirical absorption correction (North et al., 1968) was applied. Of 3049 unique reflections measured, 2421 had $F_o \geq 2\sigma(F)$. The structure was solved using the program MULTAN (Main et al., 1980) and refined by the block diagonal least-squares program. During the refinement it was noticed that the C(1)–C(2) bond distance is apparently unusually short, possibly due to a disorder. All hydrogen atoms except those attached to C(1) were located with the help of a difference electron density map as well as stereochemical considerations. Hydrogens were refined isotropically while non-hydrogen atoms were refined anisotropically. The refinement converged at an R of 0.078. The atomic scattering factors were taken from the *International Tables for X-ray Crystallography* (1968). The positional and thermal parameters of non-hydrogen atoms are listed in Table I. All the crystallographic calculations unless otherwise noted were done with an NRC set of crystallographic programs (Ahmed et al., 1973).

Raman and resonance Raman (RR) data were collected as described previously (Kumar & Carey, 1975; Carey & Sans Cartier, 1983). RR data for the solid dithio esters were collected by using 180° scattering from a cylindrical quartz sample tube spun in an NMR turbine. This procedure resulted in the appearance of quartz features near 610 cm^{-1} and from 800 to 810 and from 490 to 580 cm^{-1} in the spectra. These could often be removed by spectral subtraction techniques.

Results and Discussion

Structure of N-(p-Nitrobenzoyl)-DL-alanine Ethyl Dithio Ester. It has been shown that *N*-benzoyl-DL-alanine ethyl dithio ester takes up a B-like conformation in the crystalline phase (Angus et al., 1985) similar to that found for the gly-

Table II

bond	bond length (Å)	bond	bond angle (deg)
S(1)–C(2)	1.837 (10)	C(2)–S(1)–C(3)	102.5 (4)
S(1)–C(3)	1.715 (5)	C(4)–N(1)–C(6)	120.5 (3)
S(2)–C(3)	1.615 (4)	O(2)–N(2)–O(3)	122.0 (5)
O(1)–C(6)	1.229 (4)	O(2)–N(2)–C(10)	119.2 (5)
O(2)–N(2)	1.196 (7)	O(3)–N(2)–C(10)	118.5 (4)
O(3)–N(2)	1.218 (6)	S(1)–C(2)–C(1)	121.1 (10)
N(1)–C(4)	1.447 (5)	S(1)–C(3)–S(2)	126.3 (3)
N(1)–C(6)	1.334 (4)	S(1)–C(3)–C(4)	113.0 (3)
N(2)–C(10)	1.465 (6)	S(2)–C(3)–C(4)	120.7 (3)
C(2)–C(1)	1.235 (16)	N(1)–C(4)–C(3)	112.8 (3)
C(3)–C(4)	1.528 (6)	N(1)–C(4)–C(5)	109.1 (3)
C(4)–C(5)	1.518 (7)	C(3)–C(4)–C(5)	112.0 (4)
C(6)–C(7)	1.498 (5)	O(1)–C(6)–N(1)	122.3 (3)
C(7)–C(8)	1.379 (5)	O(1)–C(6)–C(7)	120.7 (3)
C(7)–C(12)	1.384 (5)	N(1)–C(6)–C(7)	117.0 (3)
C(8)–C(9)	1.373 (6)	C(6)–C(7)–C(8)	122.2 (3)
C(9)–C(10)	1.381 (6)	C(6)–C(7)–C(12)	118.4 (3)
C(10)–C(11)	1.369 (6)	C(8)–C(7)–C(12)	119.4 (3)
C(11)–C(12)	1.363 (6)	C(7)–C(8)–C(9)	120.5 (4)
		C(8)–C(9)–C(10)	118.5 (4)
		N(2)–C(10)–C(9)	119.0 (4)
		N(2)–C(10)–C(11)	119.2 (4)
		C(9)–C(10)–C(11)	121.9 (4)
		C(10)–C(11)–C(12)	118.8 (4)
		C(7)–C(12)–C(11)	120.9 (4)

Table III: Torsional Angles[a] (deg)

	C1	C2	S1	C3	126.6 (11)
	C2	S1	C3	S2	1.9 (5)
	C2	S1	C3	C4	−178.6 (5)
ψ'	S1	C3	C4	N1	−22.7 (4)
ϕ'	C3	C4	N1	C6	−80.6 (4)
	S2	C3	C4	N1	156.9 (3)
	S2	C3	C4	C5	−79.6 (5)
	S1	C3	C4	C5	100.7 (4)
	C4	N1	C6	C7	179.5 (3)
	C4	N1	C6	O1	0.5 (5)
	N1	C6	C7	C8	−38.4 (5)
	N1	C6	C7	C12	145.2 (4)
	O3	N2	C10	C9	−10.8 (6)
	O2	N2	C10	C9	175.9 (5)
	O3	N2	C10	C11	169.6 (5)
	O2	N2	C10	C11	−3.7 (7)

[a] Standard deviations refer to the least significant digit.

cine-based analogue (Varughese et al., 1984). In the glycine case, *N*-benzoylglycine ethyl dithio ester crystallizes as a B conformer but *N*-(*p*-nitrobenzoyl)glycine ethyl dithio ester forms the radically different conformer A, in which an approximately 150° change in the $NHCH_2$–CS torsional angle has occurred [see Figure 1 and Huber et al. (1982)]. In aqueous solution any *N*-acylglycine dithio ester exists as a mixed population of A and B conformers, but variable-temperature studies show that the B conformer has the highest

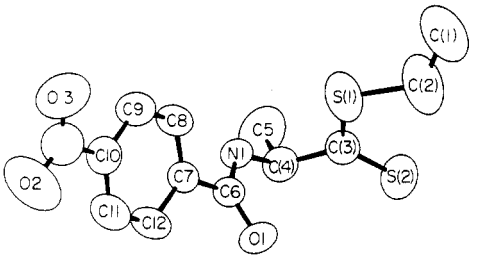

FIGURE 2: Thermal ellipsoid plot of *N*-(*p*-nitrobenzoyl)-L-alanine ethyl dithio ester.

thermodynamic stability (Storer et al., 1982). Also, in solution the RR data demonstrate that at room temperature *N*-benzoylglycine ethyl dithio ester has a higher B population compared to that of the *N*-(*p*-nitrobenzoyl)glycine dithio ester (Carey et al., 1984b). This difference in the stability of the A and B forms for the two glycine-based molecules is ascribed, in part, to the *p*-nitro group withdrawing electrons from the amide nitrogen and thus weakening the characteristic N···S (thiol) interaction found in B conformers (Carey et al., 1984b). The differential behavior of the unsubstituted and (*p*-nitrobenzoyl)glycines led us to synthesize the corresponding *N*-(*p*-nitrobenzoyl)alanine ethyl dithio ester in the hope that it would provide an A-like conformer in the solid and thus a RR spectrum–structure correlation for an alanine dithio ester in the A form. However, the crystals we obtained for *N*-(*p*-nitrobenzoyl)alanine contained the dithio ester in the B conformer. This structure will be discussed and compared to some other *N*-acylglycine and *N*-acylalanine dithio esters.

Both the D and L forms of *N*-(*p*-nitrobenzoyl)alanine ethyl dithio ester occur in the crystal. Here the discussion will focus on the L form. Under the conditions normally employed to record Raman and RR spectra (and which are used in this work), the Raman and RR spectra of the D and L forms are identical. For *N*-(*p*-nitrobenzoyl)alanine ethyl dithio ester, the crystallographic asymmetric unit contains one molecule; the coordinates for the L form are given in Table I. Bond lengths and angles are listed in Table II. The standard deviations in bond lengths vary from 0.004 to 0.007 Å except for the bonds involving C(1) or C(2). The C(1)–C(2) bond [1.24(2) Å] is unusually short, and this observed shortening is due to a disorder in the structure. The relevant torsional angles are given in Table III. Important structural parameters are compared in Table IV for the three alanine-based dithio esters presently available and for *N*-(*p*-nitrobenzoyl)glycine and *N*-benzoylglycine ethyl dithio esters. A view of the *N*-(*p*-nitrobenzoyl)alanine ethyl dithio ester is given in Figure 2. The *N*-(*p*-nitrobenzoyl)alanine ethyl dithio ester in Figure 2 is a typical B structure with $C_3C_4N_1C_6$ (ϕ') and $S_1C_3C_4N_1$ (ψ') torsional angles of −81° and −23°, respectively. There is close agreement between most of the structural parameters

Table IV: Comparison of Structural and Resonance Raman Data for Some Glycine- and Alanine-Based Ethyl Dithio Esters

ethyl dithio ester	conformer	N···S[a]	C—C[b]	C=S	C—S[c]	S—C[d]	ϕ'	ψ'	C–S–C–C	resonance Raman signature[e]
N-(*p*-nitrobenzoyl)alanine	B	2.828	1.528	1.615	1.715	1.837	−81	−23	127	1174 (m), 1144 (m), 1095 (s), 1035 (w), 678 (w), 604 (w), 564 (w)
N-benzoylalanine	B	2.830	1.530	1.614	1.718	1.807	−84.0	−23.0	−84.8	1169 (m), 1140 (m), 1091 (s), 1038 (w), 683 (w), 614 (w), 566 (w)
N-(methyloxycarbonyl)-L-phenylalanyl-L-alanine	A	3.074	1.538	1.601	1.681	1.836	−69.2	141.1	−153.9	1174 (s), 1099 (m), 1033 (w), 902 (w), 667 (m), 578 (w), 561 (w)
N-(*p*-nitrobenzoyl)-glycine	A	3.043	1.516	1.615	1.727	1.791	−86.2	−171.7	−83.9	1169 (s), 682 (m), 667 (m)
N-benzoylglycine	B	2.866	1.522	1.615	1.710	1.804	−78.7	−15.5	−176.1	1161 (w), 1118 (s), 1041 (w), 697 (w), 601 (m), 554 (w)

[a] N···S distance refers to thiol S for B conformers and to thiono (C=S) S for A conformers. [b] C—C(=S) bond. [c] C(=S)—S bond. [d] S–C_2H_5 bond. [e] S = strong, m = medium, w = weak.

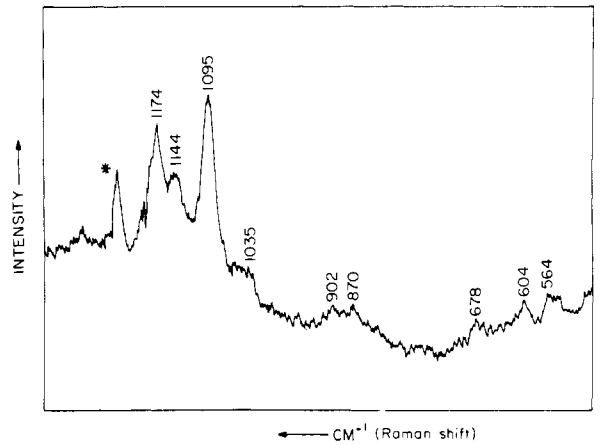

FIGURE 3: 324-nm excited RR spectrum of solid *N*-(*p*-nitrobenzoyl)-DL-alanine ethyl dithio ester. Asterisk indicates a laser plasma line.

for this compound and the alanine analogue lacking the nitro substituent; e.g., the $N_1 \cdots S_1$ distances, 2.83 Å, are the same. This latter finding is somewhat surprising since we expect the *p*-NO_2 substituent to weaken the N···S interaction, and there is some evidence that this is indeed the case for this dithio ester in solution (see below). As observed in all the other *N*-acylalanine or *N*-acylglycine dithio ester structures we have analyzed [e.g., Varughese et al. (1986, 1984) and Huber et al. (1982)], there is an intermolecular N–H···O hydrogen bond between the amide nitrogen and the carbonyl oxygen with N···O distance of 2.863(4) Å, N–H length of 0.79(4) Å, H···O distance of 2.09(4) Å, and N–H···O angle of 166(4)°.

Structure–RR Spectra Correlation for Alanine B Conformers. The RR spectrum of solid *N*-(*p*-nitrobenzoyl)alanine ethyl dithio ester, shown in Figure 3, is very similar to that for the *N*-benzoyl analogue (Figure 5, Angus et al., 1985). The intensity pattern is identical and peak positions are the same to within ±3 cm^{-1}. There were insufficient quantities of single crystals of *N*-(*p*-nitrobenzoyl)alanine ethyl dithio ester to obtain RR data for the crystalline form. However, most of the RR peaks in the spectrum seen in Figure 3, which is from the solid (prepared by removing solvent from a solution in ether), could be identified in the Raman spectrum of the single crystal (spectrum not shown). Although in the Raman spectrum of the single crystal the peak corresponding to the intense 1096-cm^{-1} feature in the RR spectrum (Figure 3) could not be seen due to the overlap with bands due to the nitro moiety (which are relatively intense in the Raman spectrum but very weak in the 324-nm excited RR spectrum), the observed correspondence between single-crystal Raman and solid RR peaks and the close similarity to the *N*-benzoylalanine case make it highly likely that the RR spectrum in Figure 3 is that of a B conformer.

Thus, we can combine the data for *N*-benzoyl-DL-alanine (Angus et al., 1985) and *N*-(*p*-nitrobenzoyl)-DL-alanine ethyl dithio esters to provide the following signature for an alanine-based dithio ester in a B form. Peak intensities are given in parentheses with the RR peak height of the most prominent feature near 1090 cm^{-1} arbitrarily defined as 10: 1169–1174 (5.5), 1140–1145 (4), 1091–1095 (10), 1035–1038 (3), 679–683 (1.5), 565–566 (1.5) cm^{-1}. There are also weak features near 610 (masked by quartz background due to the sample tube) and 890–900 cm^{-1}.

Figure 4 illustrates the RR spectrum of the solid form of *N*-(β-phenylpropionyl)-DL-alanine ethyl dithio ester unlabeled and with $^{13}C{=}S$ or CD_3 substitution. For the unlabeled molecule the RR data are so similar to those for the *N*-

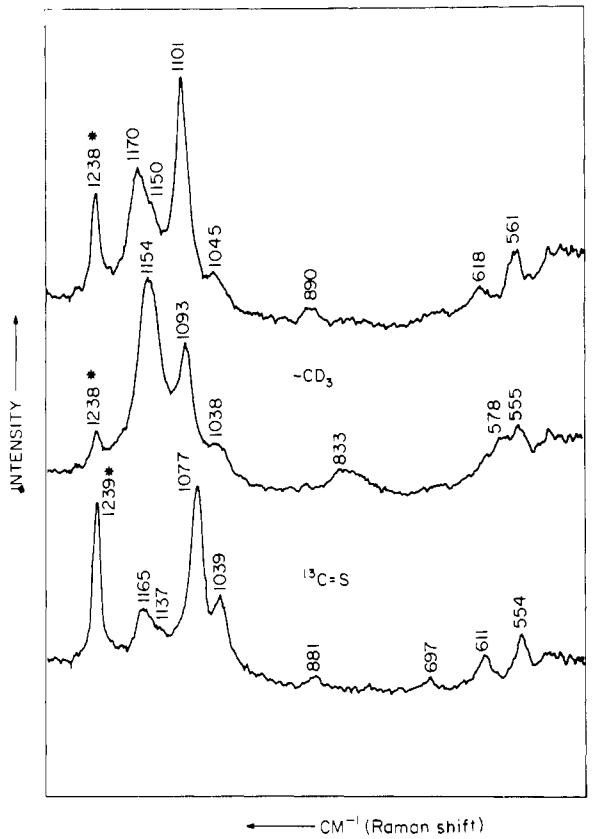

FIGURE 4: 324-nm excited RR spectra of a B-like conformer; solid *N*-(β-phenylpropionyl)-DL-alanine ethyl dithio ester and its $NHCH(CD_3)C{=}S$ (middle) and $NHCH(CH_3)^{13}C{=}S$ (bottom) analogues. Asterisk indicates laser plasma line.

benzoylalanines we may state with confidence that the *N*-(β-phenylpropionyl) analogue is solidifying as a B conformer. This conclusion is reinforced by the similar $^{13}C{=}S$ shifts seen for the *N*-(β-phenylpropionyl) compound (Figure 4) and *N*-benzoylalanine ethyl dithio ester (Angus et al., 1985). The data in Figure 4 are important since they provide the RR signatures of an alanine B conformer labeled with $^{13}C{=}S$ and, separately, with CD_3.

Major changes in the RR spectra of the B form of *N*-(β-phenylpropionyl)alanine ethyl dithio ester accompany CD_3 and $^{13}C{=}S$ substitution. In the latter case (Figure 4), there is a marked diminution of intensity in the 1170-cm^{-1} region, a shift of the most intense band from 1101 ($^{12}C{=}S$) to 1077 cm^{-1} ($^{13}C{=}S$), a shift and intensification of the band at 1045 cm^{-1} to 1039 cm^{-1}, and small shifts to lower frequency of the features seen at 890, 618, and 561 cm^{-1} in the $^{12}C{=}S$ form. For the $-CD_3$-substituted form in the solid phase, maximum intensity is observed at 1154 cm^{-1} rather than at 1101 cm^{-1} as in the $-CH_3$ case, a medium-intensity band appears at 1093 cm^{-1}, and in the 600-cm^{-1} region the 618- and 561-cm^{-1} peaks for the unlabeled compound are replaced by bands at 578 and 555 cm^{-1}.

Structure–RR Correlation for an Alanine Dithio Ester A-like Conformer. Comparison to Conformer B. Recently, a combined X-ray crystallographic and Raman spectroscopic analysis of (methyloxycarbonyl)-L-phenylalanyl-L-alanine ethyl dithio ester has provided a RR spectrum–structure correlation for an A-like conformation about the bonds near the dithio ester group (Varughese et al., 1986). For this dithio ester the S_1C_3–C_4N_1 torsional angle (ψ') is 141°, in contrast to the values (+10° to −25°) found for B conformers (Varughese et al., 1984). In the other A conformer characterized to date, *N*-(*p*-nitrobenzoyl)glycine ethyl dithio ester, ψ' was found to

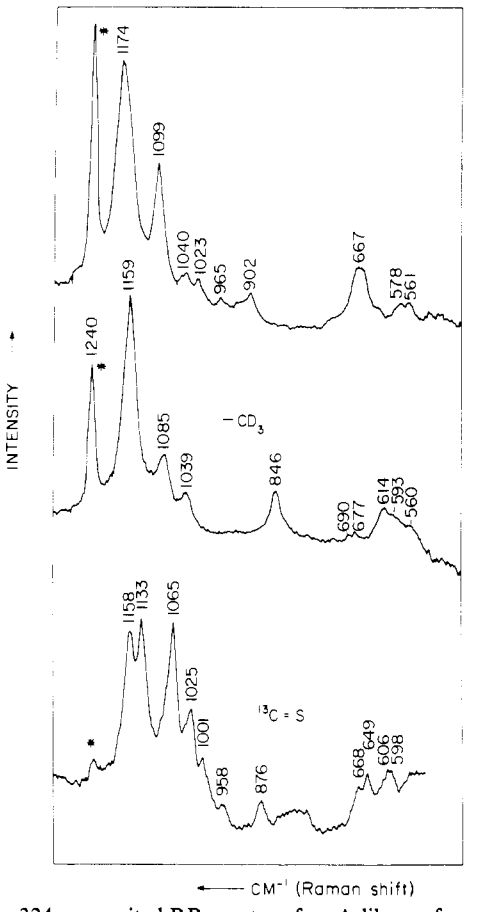

FIGURE 5: 324-nm excited RR spectra of an A-like conformer; solid (methyloxycarbonyl)-L-phenylalanyl-DL-alanine ethyl dithio ester and its $NHCH(CD_3)C{=}S$ (middle) and $NHCH(CH_3)^{13}C{=}S$ (bottom) analogues. Asterisk indicates laser plasma line.

be −172° (Huber et al., 1982). Thus, referring to Table IV, we consider A-class conformers to have ϕ' values in the −70 to −85° range and ψ' values in the +140 to −170° range, whereas B-class conformers have ϕ' values from −70 to −100° and ψ' values from +10 to −25° (Varughese et al., 1984).

The RR spectra of solid (methyloxycarbonyl)-L-phenylalanyl-L-alanine ethyl dithio ester in its unsubstituted and $NHCD_3CHC(=S)$ and $NHCH_3CH^{13}C(=S)$ forms are compared in Figure 5. The spectrum of the solid unlabeled analogue, which was prepared by removing solvent from an acetone–methylene chloride solution (1:9), is identical with the spectrum obtained from crushed single crystals (Varughese et al., 1986). Thus, the top spectrum in Figure 5 and probably the lower two are due to the A-like structure. However, two possible complications exist in the assignment of the two lower spectra. One is that an unknown amount of a non-A (probably B-like) conformer might cosolidify with the conformer A molecules. Given the spectral signatures of the B form seen in Figure 4, the amount of B in the material used to record Figure 5 must be, at most, small. The second complication concerns the coexistence of the LD and LL isomers (Varughese et al., 1986). In the single-crystal study of the unlabeled molecule, the crystals were found to contain only the LL form, and this is certainly the isomer giving rise to the top spectrum in Figure 5. However, the bottom two traces could contain contributions from the LD isomer, which, of course, is a different molecule from the LL isomer. Presently, we have no way of resolving this ambiguity but note that this is unlikely to present a problem in the solution-phase RR spectra of PheAla dithio esters since conformational differences several bonds removed from the dithio ester linkages seem to have little effect on the RR spectra; the close similarity between the RR spectra of a mixture of DL and LL PheAla and *N*-(β-phenylpropionyl)alanine ethyl dithio esters in solution provides good evidence for this assertion (compare Figures 6 and 7 with Figures 8 and 9).

For the isotopically unsubstituted molecule, the RR spectrum of the A form of the PheAla dithio ester has its most intense feature at 1174 cm^{-1} with a weaker band appearing at 1099 cm^{-1} (Figure 5). The next most intense RR feature of the A form occurs as a fairly broad band, probably containing more than one feature, at 667 cm^{-1}. This medium-intensity feature is important because B conformers give rise to a sharper and weaker band in the 680–685-cm^{-1} range, and thus a broad medium-intensity band (or doublet) near 660 is diagnostic for an A-like conformer. The A conformer of glycine-based dithio esters also has a broad band, usually a doublet, in the 660-cm^{-1} region (Huber et al., 1982). Other features in the RR spectrum of the A-like alanine conformer in Figure 5 are too weak, and usually too overlapped with B-conformer peaks, to act as conformational markers.

Upon $^{13}C{=}S$ substitution, major changes occur in the 1050–1175-cm^{-1} region, with the 1174- and 1099-cm^{-1} bands seen in the $^{12}C{=}S$ form being replaced by features at 1158, 1133, and 1065 cm^{-1} in the $^{13}C{=}S$ compound. Additionally, there is significant increase in the relative intensity of the band at 1025 cm^{-1} (Figure 5). The feature at 902 cm^{-1} in the $^{12}C{=}S$ compound probably shifts to 876 cm^{-1} in the $^{13}C{=}S$-substituted analogue. There are few discernible changes in the spectrum below 700 cm^{-1}; the "new" peak seen in the spectrum of the $^{13}C{=}S$ compound near 600 cm^{-1} is due in large part to the quartz spectrum of the sample tube.

Replacement of CH_3 by CD_3 results in the middle spectrum seen in Figure 5. The most intense peak in the solid form shifts from 1174 to 1159 cm^{-1} in the CD_3 analogue. The 1099-cm^{-1} band appears to shift to 1085 cm^{-1} and diminishes in intensity, and the broad peak at 667 cm^{-1} (CH_3) is replaced by a series of features in the 560–614-cm^{-1} region for the CD_3 compound. Additionally, a band of medium intensity appears for the A form at 846 cm^{-1} (Figure 5).

We can compare the RR signatures of alanine-based dithio esters in the A- and B-conformer states, for unlabeled or $^{13}C{=}S$- or CD_3-substituted molecules, by comparing Figures 5 and 4, respectively. Although these figures refer to different molecules, the PheAla and *N*-(β-phenylpropionyl)alanine derivatives, the fact that the RR spectra of these compounds in H_2O or CCl_4 are the same provides confidence that the RR signature of the dithio ester moiety is not affected by the chemical differences of the groups attached to the N atom of the alanine moiety. For the unlabeled molecules, going from A to B results in reversal in relative intensities in the 1174- and 1099-cm^{-1} features, with the latter being more intense in the conformer B case. The broad band at 667 cm^{-1} is characteristic for conformer A, while only the B form gives rise to a medium-intensity feature at 561 cm^{-1}.

The CD_3-substituted A and B conformers give rise to quite similar RR spectra in the 1000–1200-cm^{-1} range, although the B-conformer peak at 1093 cm^{-1} is of higher relative intensity. The A conformer has a characteristic marker at 846 cm^{-1}, replacing the broad band found in the B form at 833 cm^{-1}. With the possible exception of the A peak at 614 cm^{-1}, there are no distinct diagnostic signatures in the 600-cm^{-1} range.

The main differences in the signatures of the $^{13}C{=}S$-substituted A and B conformers are that the A has a higher relative intensity feature (a doublet) in the 1150-cm^{-1} region

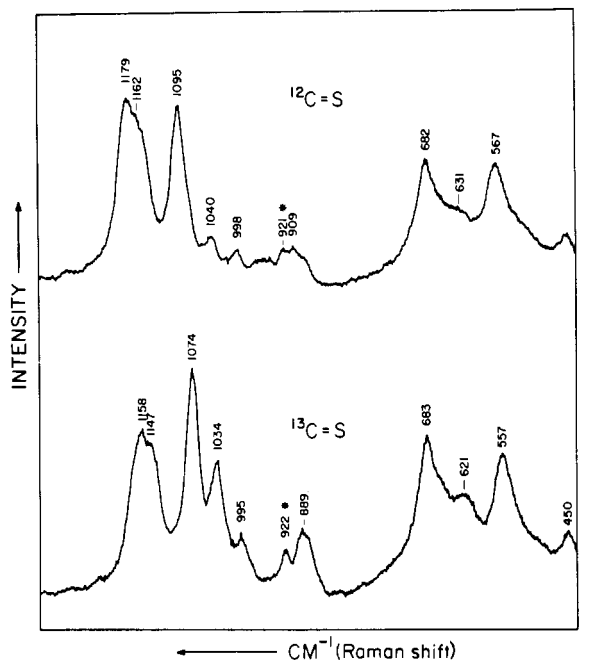

FIGURE 6: 324-nm excited RR spectra of *N*-(β-phenylpropionyl)-DL-alanine ethyl dithio ester and its ^{13}C=S-substituted analogue, 2 × 10^{-4} M in H_2O containing 1% CH_3CN. Asterisk denotes CH_3CN feature.

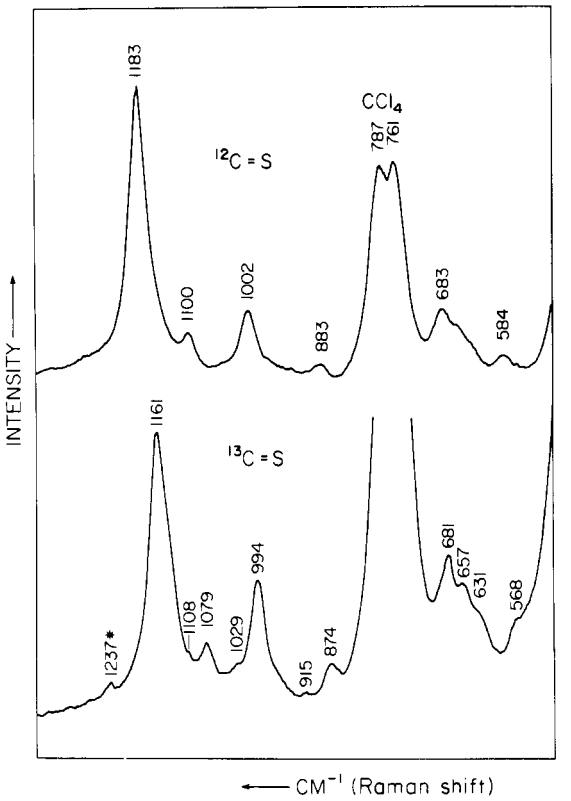

FIGURE 7: 324-nm excited RR spectra of *N*-(β-phenylpropionyl)-DL-alanine ethyl dithio ester and its ^{13}C=S-substituted analogue, 2 × 10^{-3} M in CCl_4. Asterisk denotes laser plasma line.

and the bands analogous to the 1077- and 1039-cm^{-1} bands in the B form appear at 1065 and 1025 cm^{-1} in the A conformer. The A form appears to have a weak but characteristic band at 876 cm^{-1}, while in the 500–700-cm^{-1} range the spectral differences of the ^{13}C=S-substituted molecules resemble those found for the unlabeled compounds.

The knowledge gained from solid-state studies can now be used to characterize A-like and B-like conformers of alanine dithio esters in solution.

RR Spectra of A- and B-like Conformers of Alanine Dithio Esters in Aqueous Solution and for C_5 Conformers in CCl_4. The RR spectra of *N*-benzoyl-DL-alanine ethyl dithio ester and of methyloxycarbonyl-L-phenylalanyl-DL-alanine ethyl dithio ester in solution have been discussed elsewhere (Angus et al., 1985; Varughese et al., 1986, respectively). Here we shall present data for *N*-(β-phenylpropionyl)-DL-alanine ethyl dithio ester (including its ^{13}C=S- and CD_3-labeled analogues) and for *N*-(*p*-nitrobenzoyl)alanine ethyl dithio ester. Additionally, new data for the $NHCHCD_3C(=S)$- and $NHCHCH_3{}^{13}C(=S)$-labeled phenylalanyl-L-alanine dithio ester will be discussed.

N-(β-Phenylpropionyl)-DL-alanine Ethyl Dithio Ester. The RR spectra of *N*-(β-phenylpropionyl)-DL-alanine ethyl dithio ester, in its ^{12}C=S and ^{13}C=S forms, are compared in Figures 6 and 7 for aqueous and CCl_4 solution, respectively. The solution spectra in Figures 6 and 7 can be interpreted on the basis of the "standards" set up for the A and B conformers in the crystalline state, aided by our knowledge of the behavior of *N*-acylglycine dithio esters in solution. In Figure 6 the RR spectra of aqueous *N*-(β-phenylpropionyl)-DL-alanine ethyl dithio ester are made up of contributions from A and B conformers. The profile in the ^{12}C=S spectrum in the 1162–1179-cm^{-1} region contains major contributions from A and B conformers; the profile shifts to lower frequency in the ^{13}C=S spectrum due mainly to a shift in the unresolved A peak. The 1095- and 1040-cm^{-1} peaks in the ^{12}C=S spectrum are due predominantly to B-conformer molecules. As in the RR spectrum of the solid, the 1095-cm^{-1} band shifts ≈20 cm^{-1} to lower frequency, and the band at 1040 cm^{-1} shifts to 1034 cm^{-1} and gains in relative intensity in the RR spectrum of the ^{13}C=S analogue. The peak at 567 cm^{-1} is due to B-conformer molecules and shifts to 557 cm^{-1} in the ^{13}C=S compound and compares with the 561- (^{12}C=S) to 554-cm^{-1} (^{13}C=S) shift in the RR spectra of the solid seen in Figure 4. Most of the intensity in the 630–680-cm^{-1} region in Figure 6 is due to A conformers; in general, B conformers have little or no intensity in this region. The weak peak in the ^{12}C=S and ^{13}C=S spectra near 1000 cm^{-1} suggests that a minor C_5 population is in equilibrium with the A and B conformers (see below), and the spectral intensity in the 625-cm^{-1} region supports this notion.

By analogy with *N*-acylglycine dithio esters (Huber et al., 1984), it is likely that in CCl_4 *N*-acylalanine dithio esters form C_5 conformers about the linkages near the –C(=S)S– group (Figure 1). The similarity between the two sets of RR data for the glycine- and alanine-based dithio esters strongly supports this view. Thus, in Figure 7 for the *N*-(β-phenylpropionyl) derivative we assign the intense RR peak at 1183 cm^{-1} to a mode from a C_5 conformer that shifts to 1161 cm^{-1} upon ^{13}C=S substitution. The band at 1002 cm^{-1} is found in the C_5 form of alanine-based dithio esters (Angus et al., 1985; Varughese et al., 1986), but not in the C_5 conformer of glycine-based dithio esters (Lee et al., 1983). This feature shifts slightly to lower frequency and intensifies upon ^{13}C=S substitution. In the 550–700-cm^{-1} region ^{13}C=S substitution brings about little or no change for the intensity profile with a maximum at 683 cm^{-1}, while the weak feature at 584 cm^{-1} shifts to near 570 cm^{-1}.

Good quality RR spectra of the *N*-(β-phenylpropionyl) $-CD_3$ derivative were obtained in H_2O and CCl_4 solutions. The spectra are not shown since they are essentially identical with those for the PheAla analogue in solution discussed below.

N-(p-Nitrobenzoyl)-DL-alanine Ethyl Dithio Ester. The RR spectra of *N*-(*p*-nitrobenzoyl)-DL-alanine ethyl dithio ester in CCl_4 and in H_2O (containing 2% CH_3CN) are similar to

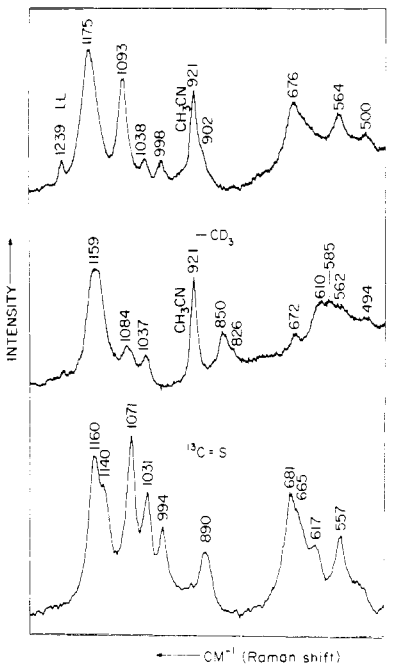

FIGURE 8: 324-nm excited RR spectra of (methyloxycarbonyl)-L-phenylalanyl-DL-alanine ethyl dithio ester and its $NHCH(CD_3)C{=}S$ (middle) and $NHCH(CH_3)^{13}C{=}S$ (bottom) analogues, $\sim 1.5 \times 10^{-4}$ M in 5% CH_3CN. Peaks marked by an asterisk are due to CH_3CN; in the bottom spectrum the solvent features have been removed by a computer subtraction technique. LL = contribution from a laser plasma line.

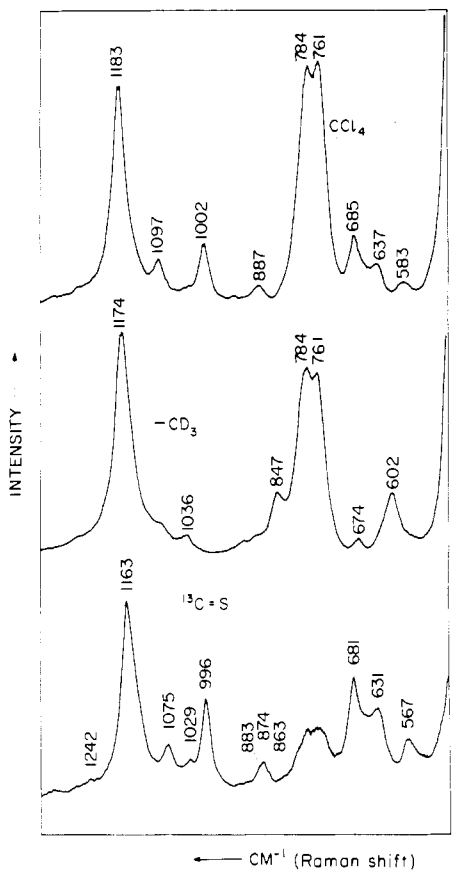

FIGURE 9: 324-nm excited RR spectra of (methyloxycarbonyl)-L-phenylalanyl-DL-alanine ethyl dithio ester and its $NHCH(CD_3)C{=}S$ (middle) and $NHCH(CH_3)^{13}C{=}S$ (bottom) analogues, 2×10^{-3} M in CCl_4. The intense doublet at 761 and 784 cm^{-1} due to CCl_4 has been effectively removed in the bottom trace by computer-aided subtraction.

those for the analogue lacking the *p*-nitro substituent (Angus et al., 1985) and for the *N*-(β-phenylpropionyl) analogue just discussed. The correspondence between the RR data for *N*-benzoyl and *N*-(*p*-nitrobenzoyl)-DL-alanine ethyl dithio esters is particularly striking. In H_2O the RR spectrum of the *p*-nitro derivative is almost identical with that of the parent compound seen in Figure 7 of Angus et al. (1985). In CCl_4 the RR spectra of the two molecules are essentially the same in the 800–1200-cm^{-1} region. In the 400–700-cm^{-1} range the peaks at 581, 635, and 696 cm^{-1} seen in Figure 8 of Angus et al. (1985) are replaced in the case of the *p*-nitro derivative by peaks at 582 (weak), 645 (medium), 674 (weak), and 719 (weak) cm^{-1}. The RR spectra of the *p*-nitro benzoyl compound contain only a very weak contribution from the symmetric stretch of the NO_2 moiety near 1350 cm^{-1} (not shown). Thus, the data for the *p*-nitro analogue can be interpreted along the lines already given in the preceding paragraphs. Interestingly, for the spectrum of the aqueous solution, the ratio of the peak intensities of the 1179- and 1094-cm^{-1} features is 1.07, compared to 1.01 for *N*-benzoyl-DL-alanine ethyl dithio ester in H_2O (Figure 7, Angus et al., 1985). The small but reproducible decrease in the relative intensity of the predominantly B feature for the *p*-nitro analogue is ascribed to a smaller B population for this molecule. As in the case of the *N*-benzoylglycine and *N*-(*p*-nitrobenzoyl)glycine ethyl dithio esters (Carey et al., 1984b) the smaller B population is caused by the *p*-nitro substituent withdrawing electrons from the amide N atoms, resulting in a weaker N···S interaction, which is one of the factors stabilizing the B conformer.

N-(Methyloxycarbonyl)-L-phenylalanyl-DL-alanine Ethyl Dithio Ester. Figures 8 and 9 compare the RR spectra for unlabeled and $NHCHCD_3CS$- and $^{13}C{=}S$-labeled (methyloxycarbonyl)-L-phenylalanyl-DL-alanine ethyl dithio ester in H_2O/CH_3CN and CCl_4, respectively. The spectra can be interpreted on the basis of our knowledge gained from the solid A and B conformers. The spectrum of the CD_3 analogue in H_2O/CH_3CN (Figure 8) is very similar to that of the solid A conformer (Figure 5). However, the analysis of the data for the unsubstituted molecule, coupled with our knowledge of similar systems, makes it highly likely that B conformers coexist with the A molecules, and this is confirmed by studying the RR spectra as a function of temperature where the peak near 1090 cm^{-1} is seen to increase in relative intensity with decreasing temperature (unpublished data from this laboratory). For the CD_3-substituted molecules the B-conformer signature is masked by peaks from A-conformer molecules. The data for the C_5 conformer of the PheAla dithio ester in CCl_4 (Figure 9) show similar trends to that for the A conformer (Figure 5). The intense band at 1183 cm^{-1} moves to lower frequency for the CD_3 form, while the 1002-cm^{-1} peak seen for the CH_3 analogue "disappears" in the spectrum of the CD_3-labeled compound. The characteristic A- (or C_5-) like band is found for the $-CD_3$ molecule at 847 cm^{-1}, and the peaks seen in the CH_3 form at 685 and 637 cm^{-1} shift to 674 and 602 cm^{-1} in the CD_3-labeled molecule (Figure 9).

For the PheAla dithio ester the effect of $^{13}C{=}S$ substitution on the RR spectra from a CCl_4 or H_2O solution can be seen in Figures 9 and 8, respectively. For the C_5 conformer in solution in CCl_4 the main change accompanying $^{13}C{=}S$ substitution is the shift of the intense 1183-cm^{-1} peak to 1163 cm^{-1} (Figure 9). The spectrum is similar to that of the $^{13}C{=}S$-substituted *N*-(β-phenylpropionyl) dithio ester in CCl_4 with the exception that the latter shows more intensity in the 657-cm^{-1} region (Figure 7). The RR spectrum of the aqueous, $^{13}C{=}S$-substituted compound (Figure 8) contains major contributions from A and B conformers and is very similar

to that of the *N*-(β-phenylpropionyl) derivative. The 1140, 1160 cm^{-1} "doublet" is due mainly to conformer A (compare the RR spectrum of the solid A conformer in Figure 5). The peaks at 890 and 681 cm^{-1} are probably also due mainly to A-like (and C_5-like, see below) conformers. The 1071- and 1031-cm^{-1} peaks contain major contributions from both conformers A and B. The 557-cm^{-1} band is due to the B population, which, on the basis of the conformer B signature seen in Figure 4, must make a substantial contribution to the intensities near 1071 and 1031 cm^{-1}.

As for the *N*-(β-phenylpropionyl) derivative, for the PheAla dithio ester in H_2O there is evidence for a feature at 998 cm^{-1} in the unsubstituted molecule that intensifies and shifts to 994 cm^{-1} in the $^{13}C{=}S$ analogue. This strongly suggests that a C_5 population is in equilibrium with the A and B conformers, and this idea gains support from the presence of the 617-cm^{-1} shoulder in the bottom ($^{13}C{=}S$) RR spectrum of Figure 8 that is in the region for a C_5 feature. Thus, C_5-like conformers can be identified in aqueous solutions of two alanine-based dithio esters we have examined here and possibly also for *N*-(*p*-nitrobenzoyl)- and *N*-benzoyl-DL-alanine ethyl dithio esters (Angus et al., 1985). This contrasts with *N*-acylglycine dithio esters, where no evidence has been found for C_5-like conformers in aqueous solution.

Another generalization to emerge from the study of alanine dithio esters, which has no precedent for *N*-acylglycine dithio esters, is the sensitivity of the RR feature near 1100 cm^{-1} to changes in the ϕ' angle. In the alanine case when this angle is in the −90° region (for an A conformer), there is a medium-intensity RR feature near 1100 cm^{-1} (Figure 5), but when $\phi' = 180°$, for the C_5 conformer predominating in CCl_4 the corresponding 1100-cm^{-1} band is very weak or missing; the weak peaks seen in the CCl_4 spectra in Figure 7 or 9 are probably due to a small B-conformer population.

Supplementary Material Available

Positional and thermal parameters of hydrogen atoms and anisotropic thermal parameters of non-hydrogen atoms (2 pages); listing of observed and calculated structure factors of *N*-(*p*-nitrobenzoyl)-DL-alanine ethyl dithio ester (14 pages). Ordering information is given on any current masthead page.

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